Investigation of the toxic & teratogenic effects of GRAS substances to the developing chicken embryo-**Citric Acid** No date

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Investigation of the Toxic and Teratogenic Effects of GRAS Substances
to the Developing Chicken Embryo
CITRIC ACID

Protocol:

Citric Acid was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by the two routes at two stages of embryonic development: via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (1, 2).

Groups of 10 or more eggs were treated under these four conditions at several dose levels until a total of ninety to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. All hatched chicks and non-viable embryos were examined carefully for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively (the milligrams per kilogram figure is based on an average egg weight of fifty grams). Column 3 is the total

number of eggs treated. Column 4 is the percent mortality i.e. total non-viable divided by total treated eggs. Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, ataxia or other nerve disorders. Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent treated eggs and the untreated controls.

The mortality data in Column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (3). The results obtained are indicated at the bottom of each table.

The data of Columns 4, 5, and 6 have been analyzed using the Chi Square Test for significant differences from the control background. Each dose level is compared to the control value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

At hatchings, 3 chicks were removed at random from each level including control for skeletal clearing, weighing and fixing of bursa, spleen, liver and kidney. Tissues were processed, blocked in paraffin, sectioned, affixed to slides, and stained. Later these sections were examined for internal damage to the tissues.

Discussion:

Citric acid was tested at dose levels between 5 and 200 mg/kg for each of the four test conditions employed. The estimated LD-50 values for all four conditions of test were as follows:

Treatment	LD-50 Level
Air cell treatment 0 hours	209.25 mg/kg (10.5 mg/egg)
Air cell treatment 96 hours	111.97 mg/kg (5.60 mg/egg)
Yolk treatment 0 hours	136.69 mg/kg (6.83 mg/egg)
Yolk treatment 96 hours	91.29 mg/kg (4.56 mg/egg)

Citric acid when administered at a dose level of 10 mg/kg or above produced significantly high mortality rates in all test conditions except for air cell treatment at 0 hours. A dose level of even a 5 mg/kg was severe in yolk treatment at 96 hours of embryonic development. The data obtained indicates that citric acid treatment would be more severe at 96 hours of embryonic treatment than the pre-incubation treatment.

No abnormalities were observed in the hatched chicks from the four different test conditions employed.

References:

1. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

2. Verrett, M. J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1003-1006.

3. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendic I.

CITRIC ACID
AIR CELL 0 HOURS

DOSE		Number of	Percent	Percent Abnormal	
mg/egg	mg/kg	Eggs	Mortality *	Total	Structural
10.00	200.00	100	58.00 *	0.0	0.0
5.00	100.00	99	41.41 *	0.0	0.0
2.50	50.00	97	29.89 *	0.0	0.0
0.50	10.00	100	18.00	0.0	0.0
0.25	5.00	100	18.00	0.0	0.0
Water		100	9.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

CITRIC ACID
AIR CELL 96 HOURS

mg/egg	mg/kg	Number of Eggs	Percent Mortality *	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	99	65.65 *	0.0	0.0
5.00	100.00	100	51.00 *	0.0	0.0
2.50	50.00	100	42.00 *	0.0	0.0
0.50	10.00	98	24.48 *	0.0	0.0
0.25	5.00	99	13.13	0.0	0.0
Water		100	10.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

CITRIC ACID
YOLK 0 HOURS

DOSE		Number of Eggs	Percent Mortality*	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.00	200.00	100	76.00*	0.0	0.0
5.00	100.00	100	55.00*	0.0	0.0
2.50	50.00	100	48.00*	0.0	0.0
0.50	10.00	100	47.00*	0.0	0.0
0.25	5.00	100	39.00	0.0	0.0
Water		100	29.00	0.0	0.0

Significantly different from solvent $p \leq 0.05$

CITRIC ACID
YOLK 96 HOURS

DOSE		Number of	Percent	Percent Abnormal	
mg/egg	mg/kg	Eggs	Mortality*	Total	Structural
10.00	200.00	100	80.00 *	0.0	0.0
5.00	100.00	99	56.55 *	0.0	0.0
2.50	50.00	99	52.52 *	0.0	0.0
0.50	10.00	100	46.00 *	0.0	0.0
0.25	5.00	100	42.00 *	0.0	0.0
Water		100	27.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$